IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(E): 2321-8851; ISSN(P): 2347-4580
Vol. 3, Issue 1, Jan 2015, 103-112

IMPACT OF NON-INVASIVE METHODS FOR DETECTION OF H. PYLORI INFECTION IN PATIENTS WITH UPPER GIT SYMPTOMS IN SOUTHERN RIYADH, SAUDI ARABIA

IBRAHIM M. ABDEL AZIZ

Department of Medicine, Gastroenterology Division, Salman Bin Abdul Aziz University Hospital, College of Medicine, Al Kharj, Saudi Arabia

Department of Gastroenterology & Infectious Diseases, Al Azhar University Hospitals, Faculty of Medicine, Cairo, Egypt

ABSTRACT

H. pylori is a ubiquitous organism. At least 50% of all people are infected, but an exact determination is not available, mostly because exact data are not available from developing countries. *H pylori* may be detected in approximately 90% of individuals with peptic ulcer disease; however, less than 15% of infected persons may have this disease. An association between H. pylori and gastric cancer is confirmed. In developing countries *H pylori* infection may be acquired at any age as gastric cancer rates are very high. According to some epidemiologic studies, this infection is acquired most frequently during childhood. Children and females have a higher incidence of reinfection (5-8%) than adult males. As well as no sex predilection is knoawn; however, females have a higher incidence of reinfection (5-8%) than males. Diagnosis of Helicobacter pylori is achieved by non-invasive methods such as H. pylori ELISA and or fecal antigen detection through ELISA techniques. The aim of the current study is to specify the impact of non-invasive methods for detection of H. pylori infection in patients with upper GIT symptoms in southern of Riyadh, as diagnostic methods in the Kingdom of Saudi Arabia.

Design & Methods

Patients presented to our GIT clinic at Salman University hospital with any of GIT symptoms such as (Heart burn, eructation, dyspepsia, and symptoms of Gastroesophageal reflux disease (GER) like, epigastric pain belching, bloating, nausea and sometimes vomiting) were enrolled from the Salman Bin Abdel Aziz University Hospital, Al Kharj, Saudi Arabia at that period between 2012-2013. Patients responded to a questionnaire to investigate possible GIT symptoms and then underwent. The stool ELISA test and H. pylori ELISA serology were applied.

Results

The diagnostic performance of the HP stool antigen assay was as follows (Table 2): sensitivity of 94.5%, specificity, 96.2%; positive-predictive value, 93%; negative- predictive value, 94%; and concordance of 90.4%. The diagnostic performance of the H. pylori ELISA assay was also high, with a sensitivity of 90.5% and specificity of 92%. Combining the HP stool antigen with the ELISA assay raised the sensitivity to 98%, the specificity to 97%, PPV to 96% and NPV to 95%.

Conclusion

The ROC curve showed a good correlation between the compared methods. The standardization of the ELISA test for the detection of H. pylori in stool specimens constitutes a non-invasive diagnostic alternative.

KEYWORDS: Helicobacter Pylori, Serology ELISA, Fecal Antigen Test

INTRODUCTION

Helicobacter pylori infection is a worldwide problem. Infection with *Helicobacter pylori* remains a major healthcare burden, with persistently high prevalence rates, especially in less-developed countries. It is the most common cause of chronic gastritis, and is strongly linked to peptic ulcer disease and gastric cancer [1]. A strong association has been reported between *H pylori* infection and gastric lymphoma and adenocarcinoma of the body and antrum of the stomach particularly lymphoid tissue lymphomas (MALTomas) [2, 3]. The association of chronic *H pylori* infection with alterations in gastric mucosal cell proliferation is recognized worldwide. Furthermore, *H pylori* infection is seemingly involved in the pathogenesis of several extra-gastric diseases, such as mucosa-associated gastro-esophageal reflux disease (GERD) [4,5], coronaritis [6], iron deficiency anemia [7]. To date, such associations are still uncertain, and causality related to these associations needs to be proved.

The most common route of *H pylori* infection is either oral-to-oral (stomach contents are transmitted from mouth to mouth) or fecal-to-oral (from stool to mouth) contact. Parents and siblings seem to play a primary role in transmission. Host status relationship as *H. pylori* have some control mechanisms able to switch the transcription of different genes on or off when needed. *H. pylori* is one of the most common bacterial infectious agents with prevalence rates ranging between (29% and 90% of cases) [8,9]. *H pylori* infection occurs more frequently in developing countries than in industrialized countries. *H pylori* strains differ in their potential to cause diseases [8,9]. Moreover, the acquisition of H. *pylori* seems to occur at higher rates in developing countries with prevalence rates that differ from one country to another and may differ between different ethnic, social, or age groups within the same country [9].

H. pylori infection can be diagnosed by either non-invasive or/ invasive methods. Non-invasive methods of detection include serology, *Helicobacter pylori* stool antigen (HPSAg) test and urea breath test (UBT) [10,11]. Invasive techniques based endoscopy includes: CLO tests, culture, histology, direct gram stain and PCR- based methods. Immunoglobulins A (IgA) and IgG serologic tests have been evaluated in several studies but the reliability and cut-off value have not been confirmed. Some studies suggested the use of IgM as an indicator of active disease [12-14] while others have found IgM to have little diagnostic utility [15,16]. The HPSAg test has been reported to be as reliable as the UBT for diagnosis as well as for monitoring *H pylori* eradication albeit at a lower sensitivity [17,18]. Although culture is considered the gold standard it is not often used for the detection of *H pylori, it* is sometime not possible to perform either due to clinical problems preventing endoscopy, or patients' refusal, or insufficient endoscopic or laboratory facilities. Among different geographical regions, the prevalence of H. pylori in the Kingdom of Saudi Arabia is between 70-90% (19). Since the prevalence of H. Pylori is still high, feasible diagnostic noninvasive tests are required for the determination of diagnosis and follow-up after eradication treatment. Therefore, it is crucial to optimize non-invasive methods for reliable diagnosis of Helicobacter pylori infection and for post-therapy assessment. Therefore, the current study is designed to analyze the utility and diagnostic performance of a panel of non-invasive tests for the diagnosis and monitoring of *H pylori*

eradication among a population with gastric manifestations in Al Kharj city, Southern of Riyadh, Saudi Arabia.

PATIENTS & METHODS

A total of 100 outpatients complaining of upper gastroduodenal disorders and dyspepsia were enrolled in this study period between 2012 -2013. At presentation no patient had previous specific therapy for *Helicobacter pylori*.

Exclusion Criteria

Patients with a history of antibiotics, proton pump inhibitors, bismuth within one month prior to serology or HPSAg test. Patients with previous gastric surgery, and previous diagnosis of gastric cancer were also excluded from the study. All patients were subjected to clinical examination and were assessed for manifestations of gastrointestinal symptoms. Informed consent was obtained from all patients prior to participation in this study and before any procedure. The Institutional Review Board (IRB) of the University of Salman Bin Abdel Aziz University approved this study. The study was conducted according to the principles of the 1974 Declaration of Helsinki.

Helicobacter Pylori Stool Antigen Assay

Stool specimens were collected and stored at −20oC upon arrival at the laboratory after DNA extraction was performed. The stool was thawed for stool antigen assay, and subsequently stored again at−20oC for future retesting. The Helicobacter pylori Antigen, EIA, Stool Test (Quest Diagnostics, New Jeresy, USA) was used according to the manufacturer's instructions. The assay was based on S-ICT, using a single monoclonal antibody against H. pylori flagellin antigens. In brief, a plastic bar was used to add 500 mg to 1 g fecal sample to a vial containing 1 mL buffer. After gentle vortexing, the fecal sample was emulsified. Two to four drops of emulsified stool sample were placed in the sample port of the test cassette. The test was interpreted after 15 min at room temperature. The appearance of a red line in the reading window indicated a positive result, with the positive control band that was also red in color. A positive result (antigen detected) is indicative of *H pylori* presence. A negative result (antigen not detected) indicates absence of *H pylori* or an antigenic level below the assay limit of detection. The test has a sensitivity and specificity of 96% for detecting *H pylori* infection. False- negative results may be obtained on specimens from patients who have ingested selected medications (antimicrobials, proton pump inhibitors, bismuth preparations) within the 2 weeks prior to specimen collection. Serology was also performed using an enzyme-linked immunosorbent assay (IgG and IgA using Helicobacter pylori IgG, ELISA Kit (MBS580106) (Biosource, Heidelberg Germany according to the manufacturer’s instructions. Breifly, Diluted attaint serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

The diagnosis of H. pylori infection was established by the concordance of 2 or more positive test results from the 2 tests performed (i.e.; HP stool antigen or HP ELISA). H. pylori infection status was classified as follows: definite positive, all 2 tests show positive results, or a one positive result and other test show a negative result; positive results by stool antigen assay and HP ELISA; and negative, both 2 tests are negative.

STATISTICAL ANALYSIS

Continuous variables were compared using Student's t -test or the Mann–Whitney U test when appropriate. The frequencies were compared using the chi-square test or the Fisher's test if the expected frequency for any cell was five or lower. Results are expressed as mean values +/- SD, P-values and 95% confidence intervals or median and IQR as appropriate. The Z test was used to compare proportions between groups. Statistical analysis was done using Statistical Analysis Software version 16 (SAS Institute, Inc, NC, USA).

RESULTS

Among the 100 enrolled patients, the prevalence of H. pylori infection in males and females was 47.9 and 59.4 respectively. The median age of the patients was 42 years (range, 26–51 years). The prevalence of H. pylori infection in patients < 40 and > 40 years was 33.3 and 55.2, respectively (p=0.04). The prevalence of H. pylori infection by disease is as follows: reflux esophagitis 15/21; peptic ulcer in 13/17 patients; intestinal metaplasia in 5/6 patient; atrophic gastritis in 19/23 patients.

Among the 100 patients, 63, 20, and 17 had definite positive, probable positive and absolute negative H. pylori infection status. The diagnostic performance of the HP stool antigen assay was as follows (Table 3): sensitivity of 94.5%, specificity, 96.2%; positive-predictive value, 93%; negative-predictive value, 94%; and concordance of 90.4%. H pylori fecal antigen test is a novel rapid test is based on monoclonal antibody immune chromatography of stool samples. The results are positive in the initial stages of infection and can be used to detect eradication after treatment. Although the *H. pylori* fecal antigen test is an interesting tool, information about the cost of the test is pending.

The diagnostic performance of the H. pylori ELISA assay was also high, with a sensitivity of 90.5% and specificity of 92%. Combining the HP stool antigen with the ELISA assay raised the sensitivity to 98%, the specificity to 97%, PPV to 96% and NPV to 95%. It is currently based on the quantitation of immunoglobulin G antibodies against *H pylori* by the means of an enzyme-linked immune sorbent assay.

It is useful for detecting a newly infected patient, but it is not a good test for follow-up of treated patients because the results do not indicate present infection with *H pylori*. The antibody titer may remain elevated for a long time after *H. pylori* eradication. The number of false-positive results is age related and increases with age.

DISCUSSIONS

The stool antigen test for H. Pylori diagnosis has been approved and standardized for use in primary diagnosis and monitoring after treatment. In addition, the Maastricht III Consensus report recommends the use of both the urea breath test and stool antigen test for the diagnosis and follow-up of H. pylori infection (20, 21). However, the stool antigen test is non- invasive, cost effective, and requires a short time to perform. Therefore, it is convenient for patients and can be easily performed even in small laboratories and primary outpatient clinics. On the other hand, the HP ELISA requires specific detection equipment which delays the diagnostic process in addition it is more expensive (22). The prevalence of H. pylori infection in the present study is similar to that in previous reports of Helicobacter pylori in the Kingdom of Saudi Arabia (23, 24). The sensitivity and specificity of the HP stool antigen are similar that of a systematic view of 89 studies, which determined sensitivities of 91 and 93 (7). Although it is recommended to test multiple stool specimens in batches (21),

only one sample was used for each patient in this study, which may have decreased the sensitivity by 5–10. Although the infection rate was high when 2 specimens were obtained, it was not significantly higher than that determined with 1, or 3 or more specimens. A previous study reported that people > 40 years of age showed higher positive rates of H. pylori infection (5). In the current study, there was significant differences between the prevalence of H. pylori in patients >40 and <40 years of age.

The diagnostic performance of the HP stool antigen assay was as follows (Table 2): sensitivity of 94.5%, specificity, 96.2%; positive-predictive value, 93%; negative- predictive value, 94%; and concordance of 90.4%. The diagnostic performance of the H. pylori ELISA assay was also high, with a sensitivity of 90.5% and specificity of 92%. H. Pylori ELISA test is useful for detecting a newly infected patient, but it is not a good test for follow-up of treated patients because the results do not indicate present infection with *H pylori*. The antibody titer may remain elevated for a long time after *H. pylori* eradication. The number of false-positive results is age related and increases with age.

Combining the HP stool antigen with the ELISA assay raised the sensitivity to 98%, the specificity to 97%, PPV to 96% and NPV to 95%. Previous studies reported that combining more than one non-invasive assay performed better. The rapid urease test, which has been reported to have low sensitivity in atrophic gastritis and intestinal metaplasia (19, 24), the present study showed 81%–100% sensitivity and specificity in cases of atrophic gastritis, intestinal metaplasia, chronic active gastritis, and intestinal metaplasia. GERD has been reported to be inversely correlated with H. pylori infection rates (24). In the present study, the prevalence of H. pylori infection showed an inverse correlation between these 2 groups, but the performance of the evaluated kit produced similar results. The inclusion of patients with ulcers may have affected the diagnostic performance of the test in the present and previous studies. Therefore, guidelines for establishing the ratio of patients with and without ulcers to be included in evaluations of stool antigen tests may be required to reduce selection bias.

Combinations of at least 2 tests have been adopted (25) including HP stool antigen, and HP ELISA. The tests used in the present study are different from those used in previous studies, including the urea breath test, culture, rapid urease test on biopsy specimens, serological testing, and histology as the gold standard. Our results confirm that HP stool antigen, and HP ELISA assays are non-invasive rapid test for H. pylori diagnosis that demonstrates high performance among patients with upper GIT symptoms.

CONCLUSIONS

Our study showed a good assessment & correlation between the non-invasive methods. The combination of the H. pylori stools antigen and H. pylori ELISA test for the detection of H. pylori in stool specimens constitutes a good non-invasive diagnostic alternative procedure.

ACKNOWLEDGEMENTS

The corresponding author Ibrahim M Abdel Aziz; extend the appreciation to all clinical physician's and lab medicine department and medical secretaries in department of medicine, Salman Bin Abdul Aziz University hospital, Alkharj, Saudi Arabia for their continuous helpful and typing this manuscript.

CONFLICT OF INTEREST

Specific Authors Contributions

Professor Ibrahim M Abdel Aziz planned and designed the study, conducted patients' recruitment, clinical assessment, follow-up and data collection, data interpretation, sampling size and drafting of the manuscript. Professor Abdel Aziz has approved the final draft submitted. Dr. Abdel Aziz willing to submit any formation needed regarding this study.

Key Point

- As we know, H.P is highly endemic & resistant to usual therapy especially in developing countries.
- Early detection & diagnosis of H. P infection it was very important to decline the most dangerous consequences e.g: (MALTomas).
- New modalities for the diagnosis of H.P both non-invasive and invasive procedures (endoscopy, PCR histology & Culture) open new hope for early detection and treatment as well for eradication of H.P.
- Quality of life will be improved by early detection and management of H.P, as well as the dyspeptic symptoms & Gastric lymphoma (MALT) will be decline.

REFERENCES

1. Versalovic, J, Carroll, K, Funke, G, et al. (2011): Manual of Clinical Microbiology. ASM Press, Washington, D.C.
2. Suerbaum, S. and Michetti, P. (2002): Helicobacter pylori infection. N. Engl. J. Med, 347, 1175–1186.
3. Hirschl, A. and Makristathis, A. (2005): Non-invasive Helicobacter pylori diagnosis: stool or breath tests? Dig. Liver Dis, 37, 732–734.
4. Blanco, S, Forne, M, Lacoma, A, et al. (2009): Evaluation of a latex agglutination test (PYLOGEN) for the detection of Helicobacter pylori in stool specimens. Diagn. Microbiol. Infect. Dis, 63, 349–353.
5. Choi, J, Kim, C, Kim, D, et al. (2011): Prospective evaluation of a new stool antigen test for the detection of Helicobacter pylori, in comparison with histology, rapid urease test, C-urea breath test, and serology. J. Gastroenterol. Hepatol, 26, 1053–1059.
6. Cirak, M, Akyon, Y. and Megraud, F. (2007): Diagnosis of Helicobacter pylori. Helicobacter, 12, 4–9.
7. McNulty, C, Lehours, P. and Megraud, F. (2011): Diagnosis of Helicobacter pylori infection. Helicobacter, 16 (Suppl.), 10–18.
8. Veijola, L, Myllyluoma, E, Korpela, R, et al. (2005): Stool antigen tests in the diagnosis of Helicobacter pylori infection before and after eradication therapy. World J. Gastroenterol.14, 7340–44.
9. Manes, G, Zanetti, M.V, Piccirillo, M.M, et al. (2005): Accuracy of a new monoclonal stool antigen test in post-eradication assessment of Helicobacter pylori infection: comparison with the polyclonal stool antigen test and urea breath test. Dig. Liver Dis, 37, 751–755.

10. Shimoyama, T, Sawaya, M, Ishiguro, A, et al. (2011):Applicability of a rapid stool antigen test, using monoclonal antibody to catalase, for the management of Helicobacter pylori infection. J. Gastroenterol, 46, 487–491.
11. Suzuki, N, Wakasugi, M, Nakaya, S. et al. (2002): Production and application of new monoclonal antibodies specific for a fecal Helicobacter pylori antigen.
12. Dominguez, J, Forne, M, Blanco, S, et al. (2006): Comparison of a monoclonal with a polyclonal antibody-based enzyme immunoassay stool test in diagnosing Helicobacter pylori Infection before and after eradication therapy. Aliment. Pharmacol. Ther, 23, 1735–1740.
13. Deguchi, R, Matsushima, M, Suzuki, T, et al. (2009): Comparison of a monoclonal with a polyclonal antibody-based enzyme immunoassay test in diagnosing Helicobacter pylori Infection after eradication therapy. J. Gastroenterol, 47, 713–716
14. Chisholm, S, Watson, C, Teare, E, et al. (2004): Non-invasive diagnosis of Helicobacter pylori infectioninadultdyspeptic patients by stool antigen detection: does the rapid immunochromatography test provide a reliable alternative to con-ventional ELISA kit? J. Med. Microbiol, 53, 532–627.
15. Trevisani, L, Sartori, S, Rossi, M, et al. (2005): Evaluation of a new rapid immunoassay for the detection of Helicobacter pylori in faeces: a prospective pilot study. Aliment. Pharmacol. Ther, 21, 485–489.
16. Goh, K, Chan, W, Seiji, S, et al. (2011): Epidemiology of Helicobacter pylori infection and public health implications. Helicobacter, 16 (Suppl.), 1–9.
17. Dixon, M, Genta, R. and Yardley, J. (1996): Classification and grading of gastritis. The updated Sydney system. International Workshop on the Histopathology of Gastritis. Am. J. Surg. Pathol, 20, 1161–1168.
18. Gurtner, C, Hirschl, A, Dragosics, B, et al. (2004): Novel real-time PCR assay for detection of Helicobacter pylori infection and simultaneous clarithromycin susceptibility testing of stool and biopsy specimens. J. Clin. Microbiol, 42, 4512–4518.
19. Kim, S, Lee, H, Lee, W, et al. (2011) New allele-specific real-time PCR system for warfarin dose genotyping equipped with an automatic interpretative function that allows rapid, accurate, and user friendly reporting in clinical laboratories. Thromb. Res.
20. Shin, C, Kim, N. and Lee, H. (2009): Validation of diagnostic tests for Helicobacter pylori with regard to grade of atrophic gastritis and/or intestinal metaplasia. Helicobacter, 14, 512–519.
21. Malfertheiner, P, Megraud, F, O' Morain, C, et al. (2007): Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus report. Gut, 56, 772–781: 22. 6464
22. Gong, Y, Sun, L, Jin, S, et al (2010): Comparative study of serology and histology based detection of Helicobacter pylori infections: a large population-based study of 7,241 subjects from China. Eur. J. Clin. Microbiol. Infect. Dis, 29, 907–911.

23. Al Faleh FZ, Ali S, Aljebreen AM, Alhammad E, Abdo AA. Seroprevalence rates of Helicobacter pylori and viral hepatitis A among adolescents in three regions of the Kingdom of Saudi Arabia: is there any correlation? Helicobacter. 2010 Dec;15(6):532-7

24. Jaber SM. The pattern of CagA and VacA proteins in Helicobacter pylori seropositive asymptomatic children in western Saudi Arabia. Saudi Med J. 2005 Sep; 26(9):1372-7.

25. Working Party of the European Helicobacter pylori Study Group (1997): Guidelines for clinical trials in Helicobacter pylori infection. Working Party of the European Helicobacter pylori Study Group. Gut, 41, S1–9

APPENDICES

Table 1: Baseline Demographic and Clinical Characteristics of Patients

Parameter	
Age (years) Mean ± SD (95% CI of the mean)	42.17±6.2 (35.86-38.48)
Females (N ;%)	37 (37)
Symptoms (heart burn, dyspepsia, epigastric pain; n, %)	78 (78)
Mean total bilirubin ± SD (mg/dl) (95% CI of the mean)	1.04±1.2 0.6 to 3.5)
Mean ALT ± SD (U/liter) (95% CI of the mean)	44.5±13.96 (12.6 to 61.37)
Mean AST ± SD (U/liter) (95% CI of the mean)	43.15±12.2 (9.94 to 64.26)

Table 2: Helicobacter Pylori Detection by Hp Stool Antigen and Elisa Method

Number of Patients	Diagnosis	HP ELISA
63	HP definite positive	+
20	probable positive	-
17	Negative	-

Table 3: Predictive Value of HP Stool Antigen and HP Elisa

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
HP stool antigen	0.94 (0.44–0.96)	0.96 (0.86–0.98)	0.93 (0.77–0.98)	.94 (0.36–0.98)
HP ELISA	0.90 (0.74–0.96)	0.96 (0.80–1.00)	0.88 (0.53–0.99)	0.87 (0.42–0.97)
HP stool antigen	0.98 (0.45-100)	0.98 (0.50 1.00)	96 (0.45-0.96)	0.95(0.67-0.99)

LIST OF ABBREVIATIONS

ELISA: Elisa immunosorbent assay

H. P: Helicobacter pylori

PCR: polymerase chain reaction

PPV: Positive predictive volume

NPV: Negative predictive volume

GERD: Gastroesophageal reflux disease

HPSAg: Helicobacter pylori stool antigen

UBT: Urea breathe test.

IgA: Immunoglobulin A.

IgG: Immunoglobulin G.

IgM: Immunoglobulin

KSA: Kingdom of Saudi Arabia